Isolation of Δ^7-Cholesten-3-one from Butterfat

THE direct reaction of butterfat with 2,4-dinitrophenylhydrazine has resulted in the identification of a number of previously undescribed carbonyl compounds[1,2] from dairy products. During the course of those investigations a 2,4-dinitrophenylhydrazone (hereafter abbreviated to DNPhydrazone) was observed with chromatographic properties similar to the aldehyde DNPhydrazones but differing in light absorption properties. Initial studies revealed that the unknown DNPhydrazone gave a positive response to the Liebermann-Burchard reaction. This communication presents some investigations in which the compound has been identified as the DNPhydrazone of Δ^7-cholesten-3-one.

500 g butterfat was dissolved in 1·5 l. of carbonyl-free hexane[3]. The solution was divided and each half was passed through a 10 g 2,4-dinitrophenylhydrazine reaction column[4]. The DNPhydrazone monocarbonyl fraction was isolated from the fat by adsorption chromatography on magnesia ('Seasorb 43') and alumina columns according to the method of Schwartz *et al.*[4]. The saturated aldehyde DNPhydrazone fraction (tan in colour) was isolated from the monocarbonyl DNPhydrazones by adsorption chromatography on a 'Seasorb 43': 'Celite 545' column using 2 per cent methanol in chloroform as the eluting solvent[5]. The DNPhydrazone giving a positive Liebermann-Burchard reaction was separated from the long-chain aldehyde DNPhydrazones on a reverse phase partition chromatographic column prepared from 8 g of dodecane mechanically ground into 15 g of silanized 'HyFlo Super-cel' and packed into the column as a slurry. The eluting solvent was acetonitrile saturated with dodecane. The sample was dissolved in the eluting solvent by gentle heating and placed on the column. Several such columns were necessary to accommodate the quantity of DNPhydrazones obtained from 500 g of milkfat. The major band following the elution of the long-chain aldehyde DNPhydrazones was collected and evaporated to near dryness on a steam bath with the aid of a stream of nitrogen. The residue was taken up in chloroform and the residual dodecane removed from the DNPhydrazone by adsorption of the latter on a 'Seasorb 43': 'Celite 545' (4 g : 8 g) column. The DNPhydrazones adsorbed on the column were washed with 50 ml. of chloroform and the grey band eluted ofrm the column with

2 per cent methanol in chloroform. The effluent containing the Liebermann-Burchard positive DNPhydrazone was evaporated to dryness under nitrogen and the parent compound regenerated from the reagent by allowing a solution of the unknown in 30 ml. acetone containing 1·2 ml. of 5 N sulphuric acid to stand overnight at room temperature. The parent compound was recovered from the acetone solution as follows. Water (5 ml.) was added to the solution, which was then evaporated on a steam bath under nitrogen to approximately 10 ml. The precipitate was recovered by extracting twice with 20 ml. portions of carbonyl-free hexane, and these extracts were combined, dried with sodium sulphate, filtered and evaporated to dryness. The residue containing the unknown was separated from the acetone DNPhydrazone resulting from the renegeration by chromatographing a 5 ml. chloroform solution of the residue on a column containing 2 g of 'Seasorb 43' and 4 g of 'Celite 545' in chloroform. The column was washed with 25 ml. of chloroform and the effluent, containing the steroid, collected. The steroid was further purified by thin-layer chromatography on silica gel *G* using 0·5 per cent methanol in benzene as developing solvent. Development and recovery of the unknown (approx. 1 mg) from the glass plates were accomplished as previously described[6]. The compound crystallized from acetone and water has a melting point of 128°–130° C. Two additional recrystallizations in the same solvent system resulted in fine needles having a melting point of 138°–140° C.

The recrystallized sample was analysed by mass spectrometry. The molecular ion at *m/e* 384 and the peak at *m/e* 271 (loss of alkyl side chain) indicated that the unknown is a mono-unsaturated keto steroid of the cholestane series. Ultra-violet analysis in absolute ethanol revealed a maximum absorption at 202 mμ, which was in keeping with the previous observation that the DNPhydrazone had a maximum absorption at 365 mμ in chloroform indicating the double bond was not conjugated to the carbonyl group.

The steroid gave a 'fast acting' reaction to the Liebermann-Burchard test, a positive Zimmermann test, a negative Salkowski test, positive Tortelli-Jaffé test and a positive selenium dioxide test. These observations were in agreement with those reported and observed for

Table 1

m/e	Percentage of peak height relative to molecular ion	
	Authentic Δ^7-cholesten-3-one	Isolated Δ^7-cholesten-3-one
217	12·5	10·6
229	25	25·5
245	16·9	17
247	14·3	16
271	50	47
369	19·5	19·1
384	100	00

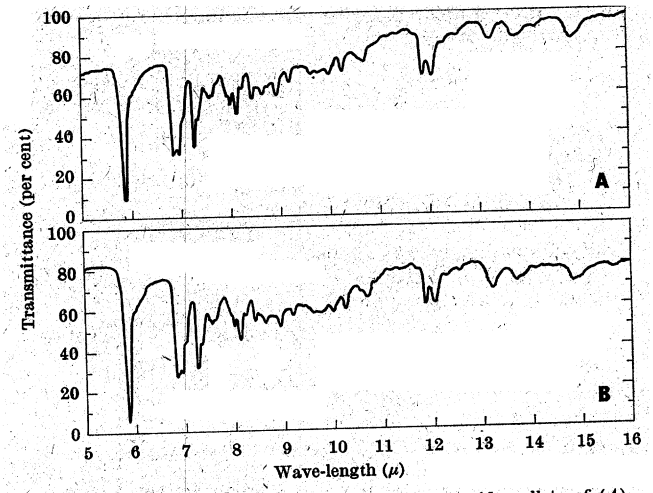

Fig. 1. Infra-red spectra of micro-potassium bromide pellets of (*A*) authentic and (*B*) isolated Δ^7-cholesten-3-one

Δ^7-cholesten-3-one prepared and purified according to Busch[7] (m.p. 145° C) (reported: 146°–148°).

Confirmation of the identification of the steroid as Δ^7-cholesten-3-one was obtained from studies of its infra-red spectra, mass spectra, and melting point. Fig. 1 is a partial reproduction of the infra-red spectra of (*A*) authentic Δ^7-cholesten-3-one and (*B*) the steroid isolated from butterfat. The identical spectra were obtained on a Beckman *IR-5A* infra-red spectrophotometer using micro-potassium bromide disks.

Table 1 is a comparison of the peak heights of the molecular fragments in the high mass range, relative to the molecular ion, of the authentic and isolated steroid. The spectra were obtained on a Bendix (model 14) 'time of flight' mass spectrometer with a vacuum locked direct inlet system (Bendix Corporation model 843*A*). A temperature of approximately 100° C was used to obtain a good quality spectra.

Recrystallization of the authentic steroid (m.p. 145°) from acetone-water, as was necessary with the isolated steroid because of the minute quantity of sample available, resulted in a lowering of the melting point to 138°–140° C. A mixed melting point with the isolated steroid (m.p. 138°–140° C) resulted in a melt at 138°–141° C. A mixed melting point between the DNPhydrazone of the authentic (m.p. 210°–212° C) and the isolated (210°–213° C) steroid crystallized from acetone–water occurred at 210°–212° C.

The presence of Δ^7-cholesten-3-one in butter fat is, so far as it is known, the only report of its occurrence in

nature. Its significance as an intermediate or by-product of cholesterol biosynthesis is speculative.

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